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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/376,395	08/18/1999	LEAF HUANG	226272002201	6461

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EXAMINER	
SCHNIZER, RICHARD A	

ART UNIT	PAPER NUMBER
1635	

DATE MAILED: 09/21/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/376,395	HUANG ET AL.	
	Examiner	Art Unit	
	Richard Schnizer, Ph. D	1635	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 June 2004*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *See Continuation Sheet* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☒ Claim(s) _____ is/are allowed.

6)☐ Claim(s) *See Continuation Sheet* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *21 June 2004* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

An amendment was received and entered on 6/21/04.

Claims 80, 81, 85, 86, 116, 118-121, 123, 135, 136, 147, 157, 158, 161, 162, and 169 were canceled and claims 178-191 were added as requested.

Claims 77, 83, 84, 88-95, 97-101, 103-115, 117, 122, 125-131, 133, 134, 137-146, 148-156, 159, 160, 163-168, and 170-191 are pending and under consideration in this Office Action.

Priority

The instant Application claims priority to two US Patents (5,795,587, filed 1,23/95; and 6,008,202, filed 9/29/97) and to abandoned application serial number 08/751,888, filed 11/18/96. Currently the application contains two claims (88 and 125), reciting compositions comprising an E1A gene. However, this embodiment finds no support in two of the three priority documents, US 5,795,587 and abandoned application serial number 08/939,874. For these reasons the effective filing date of claims 88 and 125 is 9/29/97. Similarly, support for shielding or PEG-modification of complexes is found only in US Patent 6,008,202, and not in the other two priority documents. So the priority date for claims 95, 97, 131, 133, 137, 138, 142, 143, 148-150, 154, and 155 is 9/29/97.

Drawings

The drawings filed 6/21/04 are acceptable for the purpose of examination.

Claim Objections

Applicants amendments filed 6/21/04 were sufficient to overcome the objections to claims 99, 114, and 134

Rejections Withdrawn

The rejection of claims 77, 89, 93, 94, 98-100, 104, 107-109, 139-141, 144-146, 156, 164, 168, and 172-177 under 35 U.S.C. 102(b) as being anticipated by Mack et al (Am. J. Med. Sci. 307(2): 138-143, 1992) is withdrawn in view of Applicant's amendments requiring a protamine salt.

All the rejections under 35 USC 103 have been withdrawn or reformulated as necessitated by Applicant's amendments.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 77, 83, 84, 89, 93, 94, 98-100, 103, 104, 105, 107-109, 113, 115, 117, 122, 126, 130, 134, 139-141, 144-146, 148-153, 156, 159, 160, 164, 168, 172-174, 178-186, and 188 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mack et al (Am. J. Med. Sci. 307(2): 138-143, 1992) in view of Birnstiel et al (US Patent 5,922,859, issued 7/13/99).

Mack teaches methods of preparing complexes of asialoglycoprotein-modified polylysine, plasmid DNA, and cationic lipids, and methods of using the complexes to transfect liver cells (see abstract). The complexes have a net positive charge (see page 139, column 2, third full paragraph. Absent evidence to the contrary, the positive charge is on the surface of the particles. Association of positively charged polylysine/DNA complexes with cationic lipids should reasonably result in binding of the hydrophobic portion of the lipids with the hydrophobic portions of the polylysine/DNA complex, and sequestration of these portions away from the hydrophilic medium, resulting in a positively charged surface. The ratio of nucleic acid:lipid:polycation is 1 microgram : 103 nmol : 3 microgram, assuming a molecular weight of 807 D for the cationic lipid DOGS, and use of 8.3 micrograms of DOGS. See Fig. 2 on page 140. The size of the complexes ranges from about 100 to about 2800 nanometers (see data set 'C' in Fig. 4 on page 140).

Mack does not teach the use of a protamine salt, or methods of in vivo nucleic acid delivery.

Birnstiel teaches methods and compositions for delivering DNA to cells in vitro or in vivo. The complexes comprise a polycationic polypeptide for condensing the DNA, as well as a targeting ligand ("internalizing factor"). See e.g. claims 1, 14, and 17. Birnstiel also teaches that protamine and polylysine salts may be used interchangeably in such compositions. See column 18 line 66 to column 19, line 25. The ratio of nucleic acid to polycation is an optimizable parameter as indicated at column 9, lines 18-25, but Birnstiel also exemplifies compositions comprising 6 micrograms nucleic acid and 1-3

micrograms protamine sulfate. Note that these compositions also comprised 9 micrograms of polylysine-transferrin targeting ligand conjugate. See column 18 line 66 to column 19, line 25 and Table 1 at column 27. Although Birnstiel exemplifies transferrin conjugates as an internalizing factor, the internalizing factor may also be an asialoglycoprotein such as asialotransferrin, or it may be LDL, HIV gp120, TNF, insulin, or an antibody. See column 7, lines 39-49. Birnstiel teaches that the complexes may be delivered by intratumoral injection. See column 14, lines 56-61.

Birnstiel optimized transfection efficiency by replacing some of the polylysine/transferrin complex with free polycation salts such as polylysine (55, 90, or 450 residues), protamine sulfate, or histones. First Birnstiel studied compositions comprising only transferrin/polylysine conjugates complexed with DNA, and determined the optimum amount of conjugate per unit DNA. Then Birnstiel formed complexes between DNA and a suboptimal amount of transferrins/polylysine conjugate, and then added the free polycationic polypeptide salts. Birnstiel found that "the addition of polylysines and natural protamine and the histones investigated achieved a DNA import efficiency at least equivalent to that obtained when using the conjugates which had been found to be optimum." See column 17, line 10 to column 19, line 25, especially column 18, line 47 to column 19, line 25, and Table 1 at column 27. Note that in every concentration tested, addition of free protamine sulfate provides better transfection efficiency than does addition of a free poly-L-lysine. See Table 1 at column 27. It follows that if every type of polylysine added yielded at least equivalent efficiency to that obtained with the optimum amount of conjugate in the absence of free polycation salt,

then the addition of protamine sulfate must have resulted in an improvement over that optimum.

It would have been obvious to one of ordinary skill in the art at the time of the invention to add to use protamine sulfate salts to replace some of the asialoglycoprotein-modified polylysine in the complexes of Mack, because Birnstiel demonstrates that the transfection efficiency of similar complexes is improved by this process. One of skill in the art could reasonably have expected to obtain positively charged particles in the size range of 100 to about 2800 nanometers with a mean average size of about 200-560 nanometers as shown in Fig. 4 of Mack. Furthermore, one would have been motivated to make complexes of less than 200 nm because Birnstiel teaches that efficiency of receptor-mediated uptake can be improved if the size of the complexes resembles the size of clathrin coated pits, i.e. about 100 nm. See column 4, line66 to column 5, line 16. It would have been obvious to deliver such complexes intratumorally because this is suggested by Birnstiel. See column 14, lines 56-61. Alternatively, one would have been motivated to add the lipids of Mack to the complexes of Birnstiel because Mack teaches that the addition of cationic lipids to polycation/ligand/DNA complexes increases transfection efficiency more than 10-fold over that achieved in the absence of lipids, without the need for chloroquine. See paragraph bridging columns 1 and 2, and Fig. 3, on page 140.

The instant claims require that the diameter of a claimed complex should not increase by more than 100% after 4 months of storage in 5% dextrose. A review of the specification and prosecution history shows that this performance characteristic is not

related to the use of polycation salts or protamine salts, nor to any particular method of complex formation. In fact the specification teaches that any complex of the invention is stable for up to about one year when stored at 4° C, and that the complexes can be formed by merely combining the components. See page 26, lines 19 and 20, and original claims 1 and 7. This statement regarding stability would appear to apply to the invention as originally claimed, i.e. to a drug/lipid complex comprising: at least one lipid species and a drug; the ratio of said lipid species to said drug being such that said complex has a positive charge excess of lipid to drug. Note that there is no requirement for a cationic lipid, a polycation, a polycation salt, or protamine salt in this claim, although the diameter of the particle is stable for up to a year at 4° C according to the specification. There is no apparent support for any unexpected result regarding the stability characteristics of the claimed complexes. As a result, any complex sharing the claimed structural characteristics would be expected to share the same performance characteristics. Because Birnstiel and Mack can be combined to render obvious the claimed structures, the performance characteristics of those structures are obvious as well.

Claims 88, 125, and 163 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mack (1992) and Birnstiel (1999) as applied to claims 77, 83, 84, 89, 93, 94, 98-100, 103, 104, 105, 107-109, 113, 115, 117, 122, 126, 130, 134, 139-141, 144-146, 148-153, 156, 159, 160, 164, 168, 172-174, 178-186, and 188 above, and further in view of Hung et al (US Patent 5,651,964, issued 7/29/97), Kern et al

(CANCER RESEARCH, (1990 Aug 15) 50 (16) 5184-7) and Trubetskoy et al (Biochim et Biophys. Acta 1131: 311-313, 1992).

The teachings of Mack and Birnstiel are summarized above and can be combined to render obvious compositions and methods for delivering polynucleotides to cells, in which the compositions comprise a lipid, a nucleic acid, a protamine salt, a targeting ligand, and a positive surface charge.

These references do not teach a nucleic acid encoding an E1A gene.

Hung teaches methods of suppressing growth of a neu-oncogene-mediated tumor in a mammal by delivery to the tumor of a plasmid comprising a nucleic acid sequence encoding an adenoviral E1A gene product. See claim 2.

Kern teaches that neu is overexpressed in several types of lung cancer. See abstract.

Trubetskoy teaches of antibody-modified polylysine, plasmid DNA, and cationic liposomes, and methods of using the complexes to transfect mouse lung endothelial cells (see abstract).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the composition rendered obvious by Mack and Birnstiel by using a nucleic acid encoding E1A, and to use as a targeting ligand the lung endothelial cell-specific antibody of Trubetskoy, in order to deliver the gene to mouse lung cancer cells in vivo. One would have been motivated to deliver the complexes to a lung tumor because Kern teaches that many lung tumors are characterized by overexpression of neu and because Hung teaches that growth of neu-mediated tumors can be suppressed by expression of adenoviral E1A. One would have had a reasonable expectation of success because the targeting ligand of Trubetskoy mediated successful transfection of mouse lung cells in vitro.

Claims 90, 92, 101, 106, 110-112, 127, 129, 165, 167, and 175-177 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mack (1992) and Birnstiel (1999) as applied to claims 77, 83, 84, 89, 93, 94, 98-100, 103, 104, 105, 107-109, 113, 115, 117, 122, 126, 130, 134, 139-141, 144-146, 148-153, 156, 159, 160, 164, 168, 172-174, 178-186, and 188 above, and further in view of Harris et al (US Patent 5,650,096, issued 7/22/97).

The teachings of Mack and Birnstiel are summarized above and can be combined to render obvious compositions and methods for delivering polynucleotides to cells, in which the compositions comprise a lipid, a nucleic acid, a protamine salt, a targeting ligand, and a positive surface charge.

These references do not teach the cationic lipid DC-Chol or compositions comprising neutral colipids.

Harris teaches that the cationic lipids DC-Chol and DOGS are both cationic amphiphiles useful for the delivery of nucleic acids. See column 4, lines 19-29. Harris also teaches that neutral colipids may be combined with cationic lipids to facilitate nucleic acid delivery. See brief summary paragraph 33.

MPEP 2144.06 indicates that when it is recognized in the art that elements of an invention can be substituted, one for the other, while retaining essential function, such elements are art-recognized equivalents. An express suggestion to substitute one equivalent component or process for another is not necessary to render such substitution obvious. In re Fout, 675 F.2d 297, 213 USPQ 532 (CCPA 1982). Furthermore, MPEP 2144.07 indicates that the selection of a known material based on its suitability for its intended use supports the determination of prima facie obviousness. See also Sinclair & Carroll Co. v. Interchemical Corp., 325 U.S. 327, 65 USPQ 297

(1945). In this case, both DC-chol and DOGS were recognized in the art as equivalents inasmuch as they facilitated nucleic acid delivery to cells. As such, it would have been obvious to substitute one for the other, and the invention as a whole was prima facie obvious. It would also have been obvious to include a neutral colipid in the composition because Harris taught that neutral colipids facilitate nucleic acid delivery to cells. See brief summary paragraph 33.

Claims 91, 128, and 166, are rejected under 35 U.S.C. 103(a) as being unpatentable over Mack et al (Am. J. Med. Sci. 307(2): 138-143, 1992) and Birnstiel et al (US Patent 5,922,859, issued 7/13/99) as applied to claims 77, 83, 84, 89, 93, 94, 98-100, 103, 104, 105, 107-109, 113, 115, 117, 122, 126, 130, 134, 139-141, 144-146, 148-153, 156, 159, 160, 164, 168, 172-174, 178-186, and 188, above and further in view of the evidence provided by Moir et al (J. Mol. Evol. 27 (1), 8-16 (1988)).

The teachings of Mack and Birnstiel are summarized above and can be combined to render obvious compositions and methods for delivering polynucleotides to cells, in which the compositions comprise a lipid, a nucleic acid, a protamine salt, a targeting ligand, and a positive surface charge. Birnstiel is silent as to the number of amino acids in protamine sulfate, but discloses the use of protamine sulfate from salmon.

Moir taught that salmon protamine sulfate was 33 amino acids in length, providing evidence that Birnstiel used protamine sulfate in the range of 20-100 amino acids in length. See Fig.4 "coding region". Thus the invention as a whole was prima facie obvious.

Claim 114 is rejected under 35 U.S.C. 103(a) as being unpatentable over Mack (1992) and Birnstiel (1999) as applied to claims 77, 83, 84, 89, 93, 94, 98-100, 103, 104, 105, 107-109, 113, 115, 117, 122, 126, 130, 134, 139-141, 144-146, 148-153, 156, 159, 160, 164, 168, 172-174, 178-186, and 188 above, and further in view of Wu et al (J. Biol. Chem. 263(29): 14621-14624, 1988).

The teachings of Mack and Birnstiel are summarized above and can be combined to render obvious compositions and methods for delivering polynucleotides to cells, in which the compositions comprise a lipid, a nucleic acid, a protamine salt, a targeting ligand, and a positive surface charge.

These references do not teach shielded complexes.

Wu teaches a method of intravenously delivering to cells *in vivo* complexes between the DNA and asialoglycoprotein-modified polylysine. See abstract.

It would have been obvious to one of ordinary skill in the art at the time of the invention to use the complex rendered obvious by Birnstiel and Mack to deliver the gene of Wu intravenously. One would have been motivated to do so with a reasonable expectation of success because Mack showed that the efficiency of complexes analogous to those of Wu, i.e. polycation/targeting ligand/DNA complexes, was enhanced 10-fold by addition of cationic lipids.

Claims 95, 97, 131, 133, 137, 138, 142, 143, 154, 155, and 187 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mack (1992) and Birnstiel (1999) as

applied to claims 77, 83, 84, 89, 93, 94, 98-100, 103, 104, 105, 107-109, 113, 115, 117, 122, 126, 130, 134, 139-141, 144-146, 148-153, 156, 159, 160, 164, 168, 172-174, 178-186, and 188 above, and further in view of Wu et al (J. Biol. Chem. 263(29): 14621-14624, 1988) and Torchilin et al (FASEB J. 6(9): 2716-2719, 1992).

The teachings of Mack, Birnstiel, and Wu are summarized above and can be combined to render obvious compositions and methods for intravenously delivering polynucleotides to cells, in which the compositions comprise a lipid, a nucleic acid, a protamine salt, a targeting ligand, and a positive surface charge.

These references do not teach shielded complexes.

Torchilin teaches that PEG-modification of lipidic, targeted nucleic acid complexes is advantageous for intravenous delivery because it allows prolonged circulation and avoidance of rapid clearance. See abstract.

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the complexes and methods rendered obvious by Birnstiel, Mack, and Wu by addition of polyethylene glycol. One would have been motivated to do so because Torchilin teaches that this overcomes the problem of rapid particle clearance from the bloodstream, thereby increasing the chance of accurate targeting.

Thus the invention as a whole was prima facie obvious.

Claims 170, 171, and 189-191are rejected under 35 U.S.C. 103(a) as being unpatentable over Mack (1992) and Birnstiel (1999) as applied to claims 77, 83, 84, 89, 93, 94, 98-100, 103, 104, 105, 107-109, 113, 115, 117, 122, 126, 130, 134, 139-141,

144-146, 148-153, 156, 159, 160, 164, 168, 172-174, 178-186, and 188 above, and further in view of Harris et al (US Patent 5,650,096, issued 7/22/97), Wu et al (J. Biol. Chem. 263(29): 14621-14624, 1988) and Torchilin et al (FASEB J. 6(9): 2716-2719, 1992).

The teachings of all of these references are discussed above. Mack, Birnstiel, and Wu can be combined to render obvious compositions and methods for intravenously delivering polynucleotides to cells, in which the compositions comprise a lipid, a nucleic acid, a protamine salt, a targeting ligand, and a positive surface charge. It would be obvious to modify such compositions by the addition of a neutral colipid because Harris taught that neutral colipids facilitate nucleic acid delivery to cells. See brief summary paragraph 33. it would have been obvious as well to modify the complexes by addition of polyethylene glycol. One would have been motivated to do so because Torchilin teaches that this overcomes the problem of rapid particle clearance from the bloodstream, thereby increasing the chance of accurate targeting.

Thus the invention as a whole was prima facie obvious.

Response to Arguments

Applicant's arguments filed 6/21/04 have been fully considered as they apply to the grounds of rejection set forth above, but they are not persuasive.

Applicant arguments relevant to the forgoing obviousness rejections at pages 21-23 of the response.

Applicant asserts at pages 22 and 23 that neither Mack nor Birnstiel teach complexes in which the diameter of the stored complex does not increase by more than

100% over the diameter of the complex at the time of purification, after 4 months in storage in 5% dextrose. This is unpersuasive because performance characteristics of the compositions are considered to be inherent in their structures. Because Birnstiel and Mack render the structures obvious, the performance characteristics, i.e. stability of the diameter of the complex stored in dextrose over 4 months, are obvious as well. See MPEP 2112.01.

Applicant asserts at page 22 that neither Mack nor Birnstiel teach complexes having an average diameter of less than about 200 nm. This is unpersuasive because Applicant is arguing limitations that are not in the claims. None of the rejected claims recites the term "average diameter". Instead the claims require a complex with diameter of less than 200 nm. As shown in Fig. 4, Mack clearly discloses complexes of less than 200 nm. Applicant has presented no evidence or logic to indicate that modification of the complexes of Mack by removal and replacement of some polylysine/ligand conjugates with smaller protamine sulfate groups would result in an increase in size such that the complexes were greater than 200 nm. Furthermore, one would have been motivated to make complexes of less than 200 nm because Birnstiel teaches that efficiency of receptor-mediated uptake can be improved if the size of the complexes resembles the size of clathrin coated pits, i.e. about 100 nm. See column 4, line66 to column 5, line 16.

For these reasons the rejections are deemed proper.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 77, 83, 84, 89, 90, 92-94, 98-101, 103-113, 115, 117, 122, 126, 127, 129, 130, 134, 139-141, 144-146, 148-153, 156, 159, 160, 164, 165, 167, 168, 172-186, and 188 are rejected under are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-21 of U.S. Patent No. 5,795,587, in view of Mack et al (Am. J. Med. Sci. 307(2): 138-143, 1992), and Birnstiel et al (US Patent 5,922,859, issued 7/13/99).

The claims of '587 are drawn to methods of making non-covalent nucleic acid/lipid/polycationic polypeptide complexes with a net positive charge at about pH 6-8. Absent evidence to the contrary, the positive charge is on the surface of the particles. Association of positively charged polycation/DNA complexes with cationic lipids should reasonably result in binding of the hydrophobic portion of the lipids with the hydrophobic portions of the polylysine/DNA complex, and sequestration of these portions away from the hydrophilic medium, resulting in a positively charged surface. The ratio of lipid to nucleic acid may be 0.1 nmol-200nmol lipid per 1 microgram of nucleic acid. See claim 2. The ratio of polycation to nucleic acid is about 0.01 -100 microgram polycation to 1 microgram nucleic acid. See claim 4. The complexes may comprise cationic lipids such as DC-Chol and neutral phospholipids, see claim 6. The complex may have a diameter of less than 400 nm. See claim 7. Also claimed are the compositions themselves (claims 8-14 and 21), and methods of using them to deliver nucleic acids to cells (claims 15-20) in vitro or in vivo.

The claims of '587 do not require at least one protamine sulfate or chloride salt do not recite any targeting factor, and are not required to be less than 200 nm in size.

Mack teaches methods of preparing complexes of asialoglycoprotein-modified polylysine, plasmid DNA, and cationic lipids, and methods of using the complexes to transfect liver cells (see abstract). The complexes have a net positive charge (see page 139, column 2, third full paragraph. The ratio of nucleic acid:lipid:polycation is 1 microgram : 103 nmol : 3 microgram, assuming a molecular weight of 807 D for the cationic lipid DOGS, and use of 8.3 micrograms of DOGS. See Fig. 2 on page 140. The

size of the complexes ranges from about 100 to about 2800 nanometers (see data set 'C' in Fig. 4 on page 140).

Birnstiel teaches methods and compositions for delivering DNA to cells in vitro or in vivo. The complexes comprise a polycationic polypeptide for condensing the DNA, as well as a targeting ligand ("internalizing factor"). See e.g. claims 1, 14, and 17. Birnstiel also teaches that protamine and polylysine salts may be used interchangeably in such compositions. See column 18 line 66 to column 19, line 25. The ratio of nucleic acid to polycation is an optimizable parameter as indicated at column 9, lines 18-25, but Birnstiel also exemplifies compositions comprising 6 micrograms nucleic acid and 1-3 micrograms protamine sulfate. Note that these compositions also comprised 9 micrograms of polylysine-transferrin targeting ligand conjugate. See column 18 line 66 to column 19, line 25 and Table 1 at column 27. Although Birnstiel exemplifies transferrin conjugates as an internalizing factor, the internalizing factor may also be an asialoglycoprotein such as asialotransferrin, or it may be LDL, HIV gp120, TNF, insulin, or an antibody. See column 7, lines 39-49. Birnstiel teaches that the complexes may be delivered by intratumoral injection. See column 14, lines 56-61.

Birnstiel optimized transfection efficiency by replacing some of the polylysine/transferrin complex with free polycation salts such as polylysine (55, 90, or 450 residues), protamine sulfate, or histones. First Birnstiel studied compositions comprising only transferrin/polylysine conjugates complexed with DNA, and determined the optimum amount of conjugate per unit DNA. Then Birnstiel formed complexes between DNA and a suboptimal amount of transferrins/polylysine conjugate, and then

added the free polycationic polypeptide salts. Birnstiel found that "the addition of polylysines and natural protamine and the histones investigated achieved a DNA import efficiency at least equivalent to that obtained when using the conjugates which had been found to be optimum." See column 17, line 10 to column 19, line 25, especially column 18, line 47 to column 19, line 25, and Table 1 at column 27. Note that in every concentration tested, addition of free protamine sulfate provides better transfection efficiency than does addition of a free poly-L-lysine. See Table 1 at column 27. It follows that if every type of polylysine added yielded at least equivalent efficiency to that obtained with the optimum amount of conjugate in the absence of free polycation salt, then the addition of protamine sulfate must have resulted in an improvement over that optimum.

It would have been obvious to one of ordinary skill in the art at the time of the invention to add a targeting ligand to the methods and compositions of '587 because Mack teaches that use of a targeting ligand allows delivery to specific cells that comprise a receptor for the ligand. See paragraph bridging columns 1 and 2 on page 138, and first and third full paragraphs of column 2 on page 142. In so doing one would have would have had a reasonable expectation of obtaining particles of less than 400 nm and less than 200 nm as required by new claims 178, 183, and 184. It would have been obvious to one of ordinary skill in the art at the time of the invention to add to use protamine sulfate salts as taught by Birnstiel, because Birnstiel demonstrates that transfection efficiency is improved by this process.

Alternatively, it would have been obvious to one of ordinary skill in the art at the time of the invention to add the lipids of '587 to the compositions of Birnstiel because Mack teaches that the addition of cationic lipids to polycation/ligand/DNA complexes increases transfection efficiency more than 10-fold over that achieved in the absence of lipids, without the need for chloroquine. See paragraph bridging columns 1 and 2, and Fig. 3, on page 140. Thus one of ordinary skill in the art could reasonably have expected to increase the efficiency of transfection of the complexes of Birnstiel.

The instant claims require that the diameter of a claimed complex should not increase by more than 100% after 4 months of storage in 5% dextrose. A review of the specification and prosecution history shows that this performance characteristic is not related to the use of polycation salts or protamine salts, nor to any particular method of complex formation. In fact the specification teaches that any complex of the invention is stable for up to about one year when stored at 4° C, and that the complexes can be formed by merely combining the components. See page 26, lines 19 and 20, and original claims 1 and 7. This statement regarding stability would appear to apply to the invention as originally claimed, i.e. to a drug/lipid complex comprising: at least one lipid species and a drug; the ratio of said lipid species to said drug being such that said complex has a positive charge excess of lipid to drug. Note that there is no requirement for a cationic lipid, a polycation, a polycation salt, or protamine salt in this claim, although the diameter of the particle is stable for up to a year at 4° C according to the specification. There is no apparent support for any unexpected result regarding the stability characteristics of the claimed complexes. As a result, any complex sharing the

claimed structural characteristics would be expected to share the same performance characteristics. Because the cited references can be combined to render obvious the claimed structures, the performance characteristics of those structures are obvious as well.

Claims 88, 125, and 163 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-21 of U.S. Patent No. 5,795,587, Mack (1992) and Birnstiel (1999) as applied to claims 77, 83, 84, 89, 90, 92-94, 98-101, 103-113, 115, 117, 122, 126, 127, 129, 130, 134, 139-141, 144-146, 148-153, 156, 159, 160, 164, 165, 167, 168, 172-186, and 188 above, and further in view of Hung et al (US Patent 5,651,964, issued 7/29/97), Kern et al (CANCER RESEARCH, (1990 Aug 15) 50 (16) 5184-7) and Trubetskoy et al (Biochim et Biophys. Acta 1131: 311-313, 1992).

The teachings of '587, Mack and Birnstiel are summarized above and can be combined to render obvious compositions and methods for delivering polynucleotides to cells, in which the compositions comprise a lipid, a nucleic acid, a protamine salt, a targeting ligand, and a positive surface charge.

These references do not teach a nucleic acid encoding an E1A gene.

Hung teaches methods of suppressing growth of a neu-oncogene-mediated tumor in a mammal by delivery to the tumor of a plasmid comprising a nucleic acid sequence encoding an adenoviral E1A gene product. See claim 2.

Kern teaches that neu is overexpressed in several types of lung cancer. See abstract.

Trubetskoy teaches of antibody-modified polylysine, plasmid DNA, and cationic liposomes, and methods of using the complexes to transfect mouse lung endothelial cells (see abstract).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the composition rendered obvious by '587, Mack, and Birnstiel by using a nucleic acid encoding E1A, and to use as a targeting ligand the lung endothelial cell-specific antibody of Trubetskoy, in order to deliver the gene to mouse lung cancer cells in vivo. One would have been motivated to deliver the complexes to a lung tumor because Kern teaches that many lung tumors are characterized by overexpression of neu and because Hung teaches that growth of neu-mediated tumors can be suppressed by expression of adenoviral E1A. One would have had a reasonable expectation of success because the targeting ligand of Trubetskoy mediated successful transfection of mouse lung cells in vitro.

Claims 91, 128, and 166, are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-21 of U.S. Patent No. 5,795,587, Mack (1992) and Birnstiel (1999) as applied to claims 77, 83, 84, 89, 90, 92-94, 98-101, 103-113, 115, 117, 122, 126, 127, 129, 130, 134, 139-141, 144-146, 148-153, 156, 159, 160, 164, 165, 167, 168, 172-186, and 188 above and further in view of the evidence provided by Moir et al (J. Mol. Evol. 27 (1), 8-16 (1988)).

The teachings of '587, Mack, and Birnstiel are summarized above and can be combined to render obvious compositions and methods for delivering polynucleotides to cells, in which the compositions comprise a lipid, a nucleic acid, a protamine salt, a targeting ligand, and a positive surface charge. Birnstiel is silent as to the number of amino acids in protamine sulfate, but discloses the use of protamine sulfate from salmon.

Moir taught that salmon protamine sulfate was 33 amino acids in length, providing evidence that Birnstiel used protamine sulfate in the range of 20-100 amino acids in length. Thus the invention as a whole was prima facie obvious.

Claim 114 is rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-21 of U.S. Patent No. 5,795,587, Mack (1992) and Birnstiel (1999) as applied to claims 77, 83, 84, 89, 93, 94, 98-100, 103, 104, 105, 107-109, 113, 115, 117, 122, 126, 130, 134, 139-141, 144-146, 148-153, 156, 159, 160, 164, 168, 172-174, 178-186, and 188 above, and further in view of Wu et al (J. Biol. Chem. 263(29): 14621-14624, 1988).

The teachings of '587, Mack, and Birnstiel are summarized above and can be combined to render obvious compositions and methods for delivering polynucleotides to cells, in which the compositions comprise a lipid, a nucleic acid, a protamine salt, a targeting ligand, and a positive surface charge.

These references do not teach intravenous delivery.

Wu teaches a method of intravenously delivering to cells *in vivo* complexes between the DNA and asialoglycoprotein-modified polylysine. See abstract.

It would have been obvious to one of ordinary skill in the art at the time of the invention to use the complex rendered obvious by '587, Mack, and Birnstiel to deliver the gene of Wu intravenously. One would have been motivated to do so with a reasonable expectation of success because Mack showed that the efficiency of

complexes analogous to those of Wu, i.e. polycation/targeting ligand/DNA complexes, was enhanced 10-fold by addition of cationic lipids.

Claims 95, 97, 131, 133, 137, 138, 142, 143, 154, 155, 170, 171, 187, and 189-191 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-21 of U.S. Patent No. 5,795,587, Mack (1992) and Birnstiel (1999) as applied to claims 77, 83, 84, 89, 93, 94, 98-100, 103, 104, 105, 107-109, 113, 115, 117, 122, 126, 130, 134, 139-141, 144-146, 148-153, 156, 159, 160, 164, 168, 172-174, 178-186, and 188 above, and further in view of Wu et al (J. Biol. Chem. 263(29): 14621-14624, 1988) and Torchilin et al (FASEB J. 6(9): 2716-2719, 1992).

The teachings of '587, Mack, Birnstiel, and Wu are summarized above and can be combined to render obvious compositions and methods for intravenously delivering polynucleotides to cells, in which the compositions comprise a cationic lipid, a neutral colipid, a nucleic acid, a protamine salt, a targeting ligand, and a positive surface charge.

These references do not teach shielded complexes.

Torchilin teaches that PEG-modification of lipidic, targeted nucleic acid complexes is advantageous for intravenous delivery because it allows prolonged circulation and avoidance of rapid clearance. See abstract.

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the complexes and methods rendered obvious by '587 Mack

Birnstiel, and Wu by addition of polyethylene glycol. One would have been motivated to do so because Torchilin teaches that this overcomes the problem of rapid particle clearance from the bloodstream, thereby increasing the chance of accurate targeting.

Thus the invention as a whole was prima facie obvious.

Claims 170, 171, and 189-191 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-21 of U.S. Patent No. 5,795,587, Mack (1992) and Birnstiel (1999) as applied to claims 77, 83, 84, 89, 93, 94, 98-100, 103, 104, 105, 107-109, 113, 115, 117, 122, 126, 130, 134, 139-141, 144-146, 148-153, 156, 159, 160, 164, 168, 172-174, 178-186, and 188 above, and further in view of Harris et al (US Patent 5,650,096, issued 7/22/97), Wu et al (J. Biol. Chem. 263(29): 14621-14624, 1988) and Torchilin et al (FASEB J. 6(9): 2716-2719, 1992).

The teachings of all of these references are discussed above. Mack, Birnstiel, and Wu can be combined to render obvious compositions and methods for intravenously delivering polynucleotides to cells, in which the compositions comprise a lipid, a nucleic acid, a protamine salt, a targeting ligand, and a positive surface charge. It would be obvious to modify such compositions by the addition of a neutral colipid because Harris taught that neutral colipids facilitate nucleic acid delivery to cells. See brief summary paragraph 33. it would have been obvious as well to modify the complexes by addition of polyethylene glycol. One would have been motivated to do so

because Torchilin teaches that this overcomes the problem of rapid particle clearance from the bloodstream, thereby increasing the chance of accurate targeting.

Thus the invention as a whole was prima facie obvious.

Response to Arguments

Applicant's arguments filed 6/21/04 have been fully considered as they apply to the grounds of rejection set forth above, but they are not persuasive.

Applicant arguments relevant to the forgoing obviousness rejections at pages 16-18 of the response.

Applicant asserts at pages 16 and 18 that the cited art does not teach complexes in which the diameter of the stored complex does not increase by more than 100% over the diameter of the complex at the time of purification, after 4 months in storage in 5% dextrose. This is unpersuasive because performance characteristics of the compositions are considered to be inherent in their structures. Because '587, Birnstiel and Mack render the structures obvious, the performance characteristics, i.e. stability of the diameter of the complex stored in dextrose over 4 months, are obvious as well. See MPEP 2112.01.

Applicant asserts at page 17 that the cited art does not teach complexes having an "average diameter" of less than about 200 nm. This is unpersuasive because Applicant is arguing limitations that are not in the claims. None of the rejected claims recites the term "average diameter". Instead the claims require a complex with diameter of less than 200 nm. As shown in Fig. 4, Mack clearly discloses complexes of less than

200 nm. Applicant has presented no evidence or logic to indicate that modification of the complexes of Mack by removal and replacement of some polylysine/ligand conjugates with smaller protamine sulfate groups would result in an increase in size such that the complexes were greater than 200 nm. Furthermore, one would have been motivated to make complexes of less than 200 nm because Birnstiel teaches that efficiency of receptor-mediated uptake can be improved if the size of the complexes resembles the size of clathrin coated pits, i.e. about 100 nm. See column 4, line66 to column 5, line 16.

Applicant asserted at page 14 of the response that the addition of protamine sulfate leads to an unexpected and enhanced transfection efficiency. To the extent that this assertion of unexpected results applies to any of the rejections under 35 USC 103, or the double patenting rejections, it is unpersuasive. As discussed above, Birnstiel taught that, at every concentration tested, addition of protamine sulfate salt to polylysine conjugate/DNA complexes improved transfection efficiency. See Table 1 at column 27. As a result, an improvement in transfection efficiency as a result of using protamine sulfate is not unexpected.

Conclusion

No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP

§ 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner(s) should be directed to Richard Schnizer, whose telephone number is 571-272-0762. The examiner can normally be reached Monday through Friday between the hours of 6:00 AM and 3:30 PM. The examiner is off on alternate Fridays, but is sometimes in the office anyway.

If attempts to reach the examiner by telephone are unsuccessful, the Examiner's supervisor, John Leguyader, be reached at 571-272-0760. The official central fax number is 703-872-9306. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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For all other customer support, please call the USPTO Call Center (UCC) at 800-786-9199.

DAVE T. NGUYEN
PRIMARY EXAMINER

Richard Schnizer, Ph.D.